

**Docket No. SP03-121 (0: 5275-060009)**  
**Patent**

### **AMENDMENTS TO THE CLAIMS**

This listing of claims will replace all prior versions, and listings, of the claims in the application:

#### **Listing of Claims:**

1. (original) A buffered solution for multiplexed binding assays using GPCR arrays, the solution having a composition comprising: a) a buffer reagent with a pH in the range of about 6.5 to about 7.9; b) an inorganic salt of either a monovalent or divalent species, at a concentration from about 1 mM to about 500 mM; and optionally a combination of: c) a blocker reagent at a concentration of about 0.01 wt.% to about 2 wt.% of the composition, or d) protease-inhibitor at a concentration of about 0.001 mM to about 100 mM, or both c) and d).
2. (original) The buffered solution according to claim 1, wherein said pH is in a range of about 6.8-7.8.
3. (original) The buffered solution according to claim 1, wherein said pH is about 7.4-7.5.
4. (original) The buffered solution according to claim 1, wherein when said inorganic salt is a monovalent species, said concentration of said salt is about 10-500 mM.
5. (original) The buffered solution according to claim 1, wherein when said inorganic salt is a divalent species, said concentration of said salt is about 1-50 mM.
6. (original) The buffered solution according to claim 1, wherein said composition further comprising: a labeled ligand and a target compound.
7. (original) The buffered solution according to claim 1, wherein said pH buffer is made from a solution having commonly used pH control reagents selected from Tris-HCl, HEPES-KOH, TES-NH<sub>4</sub>OH, MOPS, acetate, citrate, citrate-phosphate, sodium-phosphate, maleate, or succinate buffers.

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**Docket No. SP03-121 (045275-060009)**  
**Patent**

8. (original) The buffered solution according to claim 1, wherein said inorganic salt may be selected from NaCl, KCl, CaCl<sub>2</sub>, MgCl<sub>2</sub>, MgSO<sub>4</sub>, or MnCl<sub>2</sub>.

9. (original) The buffered solution according to claim 1, wherein said blocker reagent is a hydrophilic polymer, a biopolymer, or a water-soluble protein

10. (original) The buffered solution according to claim 9, wherein said blockers characterized as a reagent that reduces background signal and does not interfere with the binding of a target molecule with the probe receptors within a biological membrane microspot.

11. (original) The buffered solution according to claim 9, wherein said hydrophilic polymer is dextran, polyvinyl alcohol, poly (ethylene glycol), poly(anetholsulfate), poly(vinyl sulfate), CM-Dextran, dextran sulfate, beta-cyclodextrin, poly(acrylic acid), poly(sodium 4-styrene sulfonate).

12. (original) The buffered solution according to claim 9, wherein said biopolymer is poly-glutamate acid, or DNA.

13. (original) The buffered solution according to claim 9, wherein said water-soluble protein is bovine serum albumin (BSA), casein, dry milk, or wheat germ agglutinin.

14. (original) The buffered solution according to claim 1, wherein said solution is protease-free.

15. (currently amended) The buffered solution according to claim 1, wherein said protease inhibitor comprises an agent selected from the group consisting of may include EDTA, EGTA, phenyl methyl sulfonyl fluoride (PMSF), bacitracin, 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), 1,10-phenanthroline, E-64, antipain, aprotinin, benzamidinium HCl, bestatin, chymostatin, L-aminocaproic acid, N-ethylmaleimide, leupeptin, pepstatin A, phosphoramidon, trypsin inhibitor, and any combination of these.

**Docket No. SP03-121 (015275-060009)**  
**Patent**

16. (withdrawn) A buffered solution for functional assays according to a GTP-analogue-binding profile approach, the solution having a composition comprising: a) a buffer reagent with a pH in the range of about 6.5 to about 7.9; b) a divalent inorganic salt, optionally together with a monovalent inorganic salt, at a concentration from about 1 mM to about 500 mM; c) guanosine 5'-diphosphate (GDP) salt at a concentration of about 0.5 mM to about 50 mM (1-10 mM); and optionally a combination of: d) a blocker reagent at a concentration of about 0.01 wt.% to about 2 wt.% of the composition, e) protease-inhibitor at a concentration of about 0.001 nM to about 100 mM, or f) an anti-oxidant reagent at a concentration of 0.01 mM to about 100 nM.

17. (withdrawn) The solution according to claim 16, wherein said GTP-analogue includes fluorescein-GTP $\gamma$ S, Bodipy-fluorescein-GTP $\gamma$ S, Bodipy-TMR-GTP $\gamma$ S, Cy3-GTP $\gamma$ S, Cy5-GTP $\gamma$ S, Eu-GTP, <sup>35</sup>S-GTP $\gamma$ S.

18. (withdrawn) The solution according to claim 16, wherein said GDP salt is selected from a group consisting of: lithium-, sodium-, and Tris-GDP salts.

19. (withdrawn) The solution according to claim 16, wherein said anti-oxidant reagent includes sodium ascorbate, ascorbic acid, carotenoid lycopene,  $\alpha$ -tocopherol,  $\beta$ -carotene, sodium azide.

20. (withdrawn) The solution according to claim 16, wherein said anti-oxidant reagent has a concentration in a range of about 0.001 wt.% to about 0.5 wt. %

21. (withdrawn) The solution according to claim 16, wherein said pH is in a range of about 6.8-7.8.

22. (withdrawn) The solution according to claim 18, wherein said pH is about 7.4-7.5.

23. (withdrawn) The solution according to claim 16, wherein said pH buffer is made from a solution having commonly used pH control reagents selected from Tris-HCl, HEPES-KOH, TES-NH<sub>4</sub>OH, MOPS, acetate, citrate, citrate-phosphate, sodium-phosphate, maleate, or succinate buffers.

**Docket No. SP03-121 (015275-060009)  
Patent**

24. (withdrawn) The solution according to claim 16, wherein said inorganic salt may be selected from NaCl, KCl, CaCl<sub>2</sub>, MgCl<sub>2</sub>, MgSO<sub>4</sub>, or MnCl<sub>2</sub>.

25. (withdrawn) The solution according to claim 16, wherein said blocker reagent is a hydrophilic polymer, a biopolymer, or a water-soluble protein.

26. (withdrawn) The solution according to claim 22, wherein said blockers characterized as a reagent that reduces background signal and does not interfere with the binding of a target molecule with the probe receptors within a biological membrane microspot.

27. (withdrawn) The solution according to claim 22, wherein said hydrophilic polymer is dextran, polyvinyl alcohol, poly (ethylene glycol), poly(anetholsulfate), poly(vinyl sulfate), CM-Dextran, dextran sulfate, beta-cyclodextrin, poly(acrylic acid), poly(sodium 4-styrene sulfonate).

28. (withdrawn) The solution according to claim 22, wherein said biopolymer is poly-glutamate acid, or DNA.

29. (withdrawn) The solution according to claim 22, wherein said water-soluble protein is bovine serum albumin (BSA), casein, dry milk, or wheat germ agglutinin.

30. (withdrawn) The solution according to claim 16, wherein said solution is protease-free.

31. (withdrawn) The solution according to claim 16, wherein said protease inhibitor may include EDTA, EGTA, phenyl methyl sulfonyl fluoride (PMSF), bacitracin, 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), 1,10-phenanthroline, E-64, antipain, aprotinin, benzamidine HCl, bestatin, chymostatin,  $\epsilon$ -aminocaproic acid, N-ethylmaleimid, leupeptin, pepstatin A, phosphoramidon, trypsin inhibitor, and any combination of these.

32. (withdrawn) A method of reducing background signal due to non-specific binding of a labeled-ligand or GTP-analogue to a substrate surface, the method comprising: a) providing a buffered solution containing a blocker reagent; b) applying said solution to an array of GPCRs;

**Docket No. SP03-121 (015275-060009)**  
**Patent**

c) applying a second solution containing a labeled ligand or GTP-analogue, in either the absence or presence of a target compound; and d) monitoring or determining the binding of said labeled ligand to a receptor, or said GTP-analogue to a G-protein coupled with said receptor in said array.

33. (withdrawn) The method according to claim 30, wherein said method further comprises a washing and dry step before data acquisition.

34. (withdrawn) A method of reducing background signal due to non-specific binding of a labeled-ligand or GTP-analogue to a substrate surface, the method comprising: a) providing a solution containing a blocker reagent and a labeled ligand or GTP-analogue, in either the absence or presence of a target compound; b) applying said solution to a microarray of GPCRs; and c) monitoring or determining the binding of said labeled ligand to a receptor, or said GTP-analogue to a G-protein coupled with said receptor in said microarray.